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(54) HYDROXYETHYL STARCH DERIVATIVES AND THEIR PRODUCTION

(71) We, AJINOMOTO CO., INC., a corporation organized under the law of Japan, of No. 6, 1-chome, Kyobashi, Chuo-ku, Tokyo, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to certain derivatives of starch, to the production of such derivatives and to pharamaceutical compositions which include such derivatives.

Sulphates of polysaccharides such as starch, oxidized starch, cellulose, xylan and dextran have been widely studied, and some of them have been found to be suitable for use as anti-lipaemic agents. The use of such sulphates for therapeutic purposes has, however, been limited because of doubts as to clinical safety. Additionally, the chemical synthesis of the sulphates of polysaccharides is difficult because the polysaccharide tends to decompose under the reaction conditions.

Hydroxyethyl starch is presently utilized as a plasma expander, and it has been found that hydroxyethyl starch has pharmocological activity, for example anti-inflammatory, anti-lipaemic, anti-arteriosclerosis, anti-ulcer or hypocholesterolic activity.

According to one aspect of the present invention, there is provided a sulphate of hydroxyethyl starch, which can be in free acid form, in the form of a metal salt or in the form of a metal complex.

Preferably the molecular weight of the hydroxyethyl starch moiety is in the range from 1000 to 200,000, the degree of substitution of hydroxyethyl groups per anhydroglucose unit in the hydroxyethyl starch is in the range from 0.2 to 1.2, the sulphur content is in the range from 2 to 18%, by weight of the sulphate of hydroxyethyl starch, and the intrinsic viscosity of the sulphate is in the range from 0.02 to 0.5 at 30°C in water.

According to another aspect of the present invention, there is provided a process for producing the desired compound, which comprises reacting hydroxyethyl starch either with chlorosulphonic acid in pyridine, or with sulphuric acid; neutralizing the reaction solution; filtering the solution; mixing the filtrate with a water-miscible organic solvent; separating the resulting precipitate; and, if necessary, converting the sulphate from free acid form to metal salt or metal complex form.

Hydroxyethyl starch used as a starting material in the process of the present invention may be prepared, for example, by contacting waxy corn starch (with an amylopectin content of over 98% by weight) with ethylene oxide or ethylene chlorohydrin. The degree of substitution of hydroxyethyl groups in the hydroxyethyl starch is preferably in the range from 0.2 to 1.2 per anhydroglucose unit, more preferably 0.6 to 1.2, to avoid rapid decomposition *in vivo*.

The instrinsic viscosity of such hydroxyethyl starch at 30°C in water is 0.02 to 0.5.

The presently preferred processes for producing the products of the present invention will now be described. They comprise reaction between the hydroxyethyl starch and either concentrated sulphuric acid, or chlorosulphonic acid in pyridine.

When concentrated sulphuric acid is used, it is found that if, for example, more than 70% sulphuric acid is used, a reaction temperature of less than 0°C must be employed to limit discoloration. If sulphuric acid having a concentration of more than 85% is used, a reaction temperature of from −30°C to −25°C is generally preferred in order to limit the production of low molecular weight products. If, however, such low molecular weight products are desired, a reaction temperature of in the range from −10°C to 0°C should be employed. With 60% to 70% sulphuric acid a reaction temperature of from 0°C to −30°C is preferably employed. Increased temperatures of about 0°C may be employed to increase the rate of reaction.

The selected hydroxyethyl starch is mixed with the sulphuric acid generally in a ratio of from 20 to 50 g of hydroxyethyl starch per 100 ml of total volume. The mixture is

stirred at the selected temperature generally for from 20 minutes to 4 hours.

At the end of the reaction period, the mixture is poured into ice water, neutralized, for example with Na_2CO_3, and filtered. The treatment with the alkaline reagent is preferably effected promptly. The filtrate is preferably concentrated to a concentration of from 3 g to 20 g of products per 100 ml of concentrate, suitably by use of a "Diafilter" (trademark of Nippon Koshinku Co., Inc.) equipped with an appropriate molecular weight membrane. The desired products can be precipitated by the addition of a water-miscible organic solvent such as methanol, ethanol or acetone. The process provides products of high purity.

In an alternative production process calcium carbonate is added to the reaction mixture (instead of Na_2CO_3), and insoluble $CaSO_4$ is removed. The sulphate of hydroxyethyl starch can be converted to the sodium salt by adding Na_2CO_3 to the solution containing the sulphate of hydroxyethyl starch. The salt can be precipitated by the addition of an alkanol. This technique is not preferred, however, because of the difficulty of removing inorganic materials.

The thus obtained reaction products can then be washed, for example, with methanol followed by acetone and ether, and then dried completely. Care should be taken to ensure that as much as possible of the calcium is replaced by sodium; otherwise the thus obtained sulphates of hydroxyethyl starch tend to decompose in a few days.

If the product is formed by the reaction of hydroxyethyl starch with chlorosulphonic acid in pyridine, then generally from 0.5 to 3 moles of chlorosulphonic acid are used per mole of anhydroglucose units of hydroxyethyl starch, and generally from 3 to 10 ml of pyridine are used per gram of hydroxyethyl starch.

To carry out the reaction, the following procedure can be employed; pyridine is cooled to 0 to −20°C, and the chlorosulphonic acid is added dropwise while maintaining the temperature of the reaction solution below 10°C. The resulting solid pyridinium salt of chlorosulphonic acid is dissolved by warming it at 50°C to 70°C, and the hydroxyethyl starch is added to the dissolved pyridinium salt. The mixture is then stirred for from 20 minutes to 2 hours.

The solubility of the hydroxyethyl starch in pyridine permits the sulphation reaction to proceed smoothly with no troublesome increase of viscosity.

At the end of the reaction, water is added to the mixture. The addition of an alcohol such as methanol in a volume ratio of 3 to 5 ml of methanol per 1 ml of the mixture precipitates the desired product as a white powder. The precipitate can then be separated by filtration, washed and dried in the same manner as described with respect to the sulphuric acid procedure.

The precipitation of the product can be improved by the addition of a saturated, aqueous salt solution containing, for example, sodium acetate or sodium chloride. This procedure increases the rate of precipitation of the product. The use of a saturated aqueous sodium acetate solution is especially preferred.

The acid form of the sulphate may be converted to a wide variety of metallic salts or complexes by the addition of selected metal salts, for example sodium carbonate, dihydroxy aluminum chloride and calcium dichloride. These metallic products have the same potential use as the free sulphates. Examples of suitable metals include sodium, potassium, lithium, magnesium, calcium, barium, aluminum, iron and bismuth.

The sulphates of hydroxyethyl starch obtained in accordance with the process of the present invention preferably have a sulphur content of from 2% to 18% by weight and a low viscosity which makes them readily adaptable for preparation in dosage unit form.

The intrinsic viscosity of the sodium sulphate salts of the present invention is generally from 0.02 to 0.5 at 30°C. The average molecular weight is from about 1000 to 200,000 (determined by gel permeation chromatography, Waters Associates ALC/GPL 501 in comparison with standard samples of dextran of several molecular weights). The molecular weight of the products of the present invention will vary widely, depending on a variety of factors, principally the reaction conditions and the molecular weight of the hydroxyethyl starch starting material. The active compounds generally have average molecular weights in the range of from 1000 to 200,000.

The anti-coagulant activity of the compounds of the present invention is about equal to that of heparin. A particular advantage, however, is that the compounds of the present invention can be prepared at relatively low cost; also they strongly inhibit both pepsin and hyaluronidase activity.

The products of the present invention inhibit hemolysis of erythocytes, accelerate erythocyte sedimentation, and inhibit heat denaturation of serum and food protein. They are useful in pharmaceutical agents, especially because of their anti-inflammatory, anti-lipaemic, anti-arteriosclerosis, anti-ulcer, and hypocholesterolic activity. They are also useful in the fields of food and chemical products.

Inhibition of heat denaturation of serum proteins is widely used for screening potential pharmaceutical agents for anti-inflammatory activity. The test is described in "Inhibition

of Heat Denaturation of Serum Protein", Y. Mizushima, Arch. Int. Pharmacodyn. *149*, 1 (1964).

In the test, the sodium salt of hydroxyethyl starch sulphate was dissolved in 1/15M phosphate buffer solution (pH=5.4) and to resulting the solution the same phosphate buffer solution containing 1% bovine serum albumin was added in a 1:1 volume ratio. The combined solution was stirred at 25°C for 20 minutes and then stirred at 67°C for 3 minutes and cooled. The turbidity of the test solutions thus obtained was compared to that of a solution obtained by adding 1/15*M* phosphate buffer solution containing 1% bovine serum albumin to 1/15*M* phosphate buffer solution in a 1:1 volume ratio, and the degrees of inhibition of heat denaturation were calculated. The results are shown in the following Table I.

TABLE 1

Sulphur Content of Sodium Salt of Sulphate of Hydroxyethyl Starch %	Concentration of Bovine Serum Albumin in Solution for Test (g/dl)	Concentration of Sodium Salt of Sulphate of Hydroxyethyl Starch in Solution for Test (M)*	Inhibition Ratios of Heat Denaturation of Bovine Serum Albumin (%)
1.6	0.5	3×10^{-3}	97.9
6.3	0.5	3×10^{-3}	98.1
8.2	0.5	3×10^{-3}	98.1
11.1	0.5	3×10^{-3}	97.6
14.5	0.5	3×10^{-3}	98.8
17.2	0.5	3×10^{-3}	93.4
(Control: phenylbutazone)	0.5	1×10^{-3}	88.8

*M; per repeating unit

As clearly shown in Table 1, the products in accordance with the present invention manifest good inhibition activities of heat denaturation of bovine serum albumin.

The potent anti-inflammatory activity of the products of the present invention was determined by the following assay.

Intact, male Sprague-Dawley rats, each weighing about 170—200 g, were randomly grouped in groups of 10, and treatment with the test compounds begun. The test compounds were suspended in physiological saline containing a few drops of Polysorbate 80, and administered subcutaneously or intragastrically. A control group of rats was simultaneously treated (but, with some rats, using a control compound instead of a compound according to the present invention, and, with other rats, using the same suspending medium but without the control compound).

One day after the administration of the compound, the animals were injected intradermally at the base of the tail with 0.6 mg. of dry, heat-killed *Mycobacterium butyricum* suspended in 0.05 ml of paraffin oil. Administration of the test compound was continued daily for an additional 19 consecutive days with the control group being similarly treated, except for the test compound. The rats were sacrificed on the 20th day and the degree of swelling in the hind paws determined by a volume displacement apparatus or by ankle circumference measurement. Each treated group was compared statistically with the control group. A compound was rated active if it caused significant reduction in swelling ($P<0.05$) compared to the results obtained with the controls (Wilcoxon rank-sum).

The compounds according to the present invention also possess the ability to lower the level of cholesterol and phospholipids in rats caused by the injection of cholesterol or Triton. The word "Triton" is a Registered Trade Mark.

The pepsin inhibition activity of the compounds of the present invention was tested. The results are listed in the following Table 2. The anti-ulcur activity of the compounds of the present invention is evident.

TABLE 2

Sulphur Content %	Pepsin Inhibition Ratio* %
17.2 (Ex. 6)	83
13.46 (Ex. 12)	46
15.48 (Ex. 13)	74
11.84 (Ex. 14)	62
Hydroxyethyl Starch	1

*0.30% Sulphate of hydroxyethyl starch aqueous solution (pH=1.8) 1 ml; 20μg pepsin aqueous solution (pH=1.8) 1 ml; and 1.5% Bovine hemoglobin (pH=1.8) 1 ml; 30°C, 10 min.

Hyaluronidase inhibition activities of the sulphate of hydroxyethyl starch were tested. The results are listed in the following Table 3. These results indicate the usefulness of the compounds of the invention as anti-lipaemic agents.

TABLE 3

Sulphur Content %	Inhibition Ratio* %
12.61 (Ex. 3)	74
17.2 (Ex. 6)	96
4.25 (Ex. 11)	53
13.46 (Ex. 12)	21
15.48 (Ex. 13)	32
11.84 (Ex. 14)	38
Hydroxyethyl Starch	10

*0.16% Sulphate of hydroxyethyl starch phosphate buffer (pH=7.0) 0.25 ml; hyaluronic acid phosphate buffer (pH=5.3) 0.5 ml; and Bovine serum albumin phosphate buffer (pH=3.75) 5.0 ml 38°C, 45 minutes.

The pharmaceutical compositions of the present invention may be orally or parenterally administered in association with conventional pharmaceutically-accepted solid or liquid diluents or carriers.

Solid compositions for oral administration include, for example, compression tablets, pills, capsules, sugar-coated pills and granules. For preparing such solid compositions, the selected sulphate of hydroxyethyl starch, which is normally the principal active ingredient, is mixed with a conventional pharmaceutical carrier, for example calcium carbonate, lactose, sucrose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives and gelatin. Wetting agents, for example, magnesium stearate or a polyethylene glycol, may be added.

Liquid compositions for oral administration include pharmaceutically-acceptable emulsions, solutions, suspensions and syrups containing inert diluents, for example water and liquid paraffin which are usually employed in the art. In addition to inert diluents, adjuvants such as wetting agents, suspending agents, sweeteners and aromatic flavouring agents may be employed.

For parenteral administration, the selected product may be dissolved in sterilized water for injection and the resulting solution introduced into an ampoule in the amount sufficient for injection, and sealed.

Because of their wide spectrum of pharmacological activities, the compounds of the present invention can be provided in a range of dosage unit forms in which the concentrations are varied to suit the particular condition under treatment. The physician or veterinary surgeon can easily select the optimum dosage unit form and dosage considering such factors as age and condition of the patient and the malady under treatment.

The optimum dosage may vary with the proposed method of administration. For example, when the compounds of the present invention are utilized as anti-inflammatory agents, the daily oral administration may vary from 20 to 10,000 mg (preferably from 50 to 2000 mg; more preferably from 500 to 2500 mg); whereas for parenteral administration, the daily dosage may be from 20 to 5000 mg (preferably from 50 to 2000 mg; more preferably from 200 to 2000 mg).

The following non-limiting Examples are given to illustrate the present invention.

Example 1

7 G of hydroxyethyl starch (Substitution degree of the hydroxyethyl group=0.60,

$$[\eta]_{H_2O}^{20}=0.324)$$

were rapidly added to 30 ml of conc. sulphuric acid (98%) at −25°C in a three-necked distillation flask. The mixture was vigorously stirred at a temperature of −25°C to −30°C for 1 hour and 35 minutes. Stirring was difficult because the mixture became progressively more viscous. On completion of the reaction, the reaction mixture was poured onto 500 g of ice, and then 70 g of calcium carbonate were added. To the resulting mixture 100 ml of water were added, to decrease the viscosity of the mixture. The pH of the resulting solution was 6. The precipitates which formed were separated by filtration through a glass filter and washed with 370 ml of water. The filtrate and washing liquid were combined to provide a solution having a volume of 850 ml. A total of 300 ml of ethanol was added to that solution, and the mixture was allowed to stand overnight at 0°C. The mixture was then filtered and 25 g of sodium carbonate were added to adjust the pH of the filtrate to 10.8. Then sufficient acetic acid was added to adjust the pH of the solution to 7.0. The filtrate was concentrated to a volume of 100 ml, and 500 ml of ethanol were added to the concentrate in order to precipitate the desired product as a white precipitate. It was separated by filtration and washed with 100 ml of ethanol (twice), 100 ml of acetone and finally with 100 ml of diethyl ether. It was dried under reduced pressure to provide 13.12 g of sodium salts of sulphates of hydroxyethyl starch.

Sulphur content: 8.20%,

$$[\eta]_{H_2O}^{20}=0.020$$

Example 2

At total of 30 ml of conc. sulphuric acid were placed in a 3-necked distillation flask

and cooled to −25°C to form a paste. To the sulphuric acid were added 10 g of hydroxyethyl starch (substitution degree of hydroxyethyl group=0.75,

$$[\eta]_{H_2O}^{30}=0.084$$

The mixture was stirred at −37°C to −23°C for 1.5 hours. The reaction mixture became viscous but smooth stirring was possible.

On completion of the reaction, the reaction mixture was poured into 500 g of ice, and then 60 g of sodium carbonate and 15 ml of water were added to adjust the pH of the solution to 7.0. The precipitate which formed was separated by filtration and washed. The filtrate and washing solution were combined to form a solution having a volume of 760 ml. A total of 190 ml of ethanol was added to this solution and the resulting solution was left standing overnight at 0°C. Then the solution was filtered, and to the filtrate 13 ml of an aqueous solution saturated with sodium carbonate were added to adjust the pH of the solution to 10.4. Thereafter the solution was treated in the same manner as that described in Example 1, to provide 12.5 of sodium salts of sulphates of hydroxyethyl starch.

Sulphur content: 8.20%,

$$[\eta]_{H_2O}^{30}=0.084$$

Example 3

10 grams of hydroxyethyl starch (substitution degree of hydroxyethyl group=0.94,

$$[\eta]_{H_2O}^{30}=0.118)$$

were reacted with 30 ml of conc. sulphuric acid in the same manner as that described in Example 2. The reaction mixture was treated in the same manner as that described in Examples 1 and 2, using 60 g of calcium carbonate, 18 ml of saturated aqueous sodium carbonate solution and 0.8 ml of acetic acid. The product weighed 17.7g.

The product was dissolved in 200 ml of water and filtered through a "Diafilter" having a membrane acting as a sieve allowing the passage therethrough of compounds having a molecular weight of up to 10,000 using 4 litres of water for de-salting, and fractionation according to molecular weight. The resulting solution was concentrated to 100 ml, and 500 ml of methanol were added. The resulting white precipitate was separated by filtration and washed in the same manner as described above. 14.3 grams of purified sodium salts of sulphates of hydroxyethyl starch were obtained.

Sulphur content: 12.61%,

$$[\eta]_{H_2O}^{30}=0.058.$$

Example 4

60 Ml of conc. sulphuric acid were cooled to −30°C. It became very viscous. To the sulphuric acid were added 20 g of hydroxyethyl starch (substitution degree of hydroxyethyl group=0.60,

$$[\eta]_{H_2O}^{30}=0.324).$$

The mixture was vigorously stirred at between −25°C to −30°C for 1.5 hours. On completion of the reaction, 200 ml of water and 1 kg of ice were added. To the resulting mixture were added 120 g of sodium carbonate powder to adjust the pH of the solution to 7.4. The mixture was filtered and the pH of the filtrate was adjusted to 7.1. 1.3 litres of solution were put into a "Diafilter" as described above and concentrated. The material in the filter was washed with 12 litres of water. The solution thus obtained was concentrated to 100 ml. To the solution 500 ml of methanol were added, and the precipitate which formed was separated by decantation centrifugation and filtration, and dried. The products (sodium salts of sulphates of hydroxyethyl starch) weighed 13.8 g.

Sulphur content: 14.45%,

$$[\eta]_{H_2O}^{30}=0.067.$$

Example 5

50 ml of 60% (by weight) sulphuric acid were placed in a three-necked flask and cooled to 0°C. To the sulphuric acid were added 10 g of the hydroxyethyl starch used in Example 4. The mixture was stirred at 0°C for three hours. On completion of the reaction, the reaction mixture was poured into 500 g of ice, and then 70 g of sodium carbonate were added to adjust the pH to 8.8. The pH was then adjusted to 7.1 with acetic acid. The resulting 600 ml of solution were put into a "Diafilter" as described above and the material in the filter was washed with 6 litres of water. The filtrate thus obtained was concentrated to 100 ml, and to the concentrate were added 500 ml of methanol; the resulting mixture was treated in the same manner as that described in Example 4. The obtained product (sodium salts of sulphates of hydroxyethyl starch) weighed 7.4 g.

Sulphur content: 1.56%,

$$[\eta]_{H_2O}^{30}=0.18.$$

Example 6

75 ml of pyridine were placed in a three-necked flask and cooled to −10°C; then

5.4 ml of chlorosulphonic acid were slowly added at between 5 to −10°C. After the addition of the chlorosulphonic acid, were added 9 g of the same hydroxyethyl starch as used in Example 3. The mixture was stirred for 45 minutes in a water bath of 70°C. The reaction mixture remained fluid and produced only a small amount of precipitate. On completion of the reaction, 100 ml of water were added followed by 875 ml of methanol and 40 ml of a 20% aqueous sodium acetate solution to produce a white precipitate which was separated by filtration, washed and dried. The product (sodium salts of sulphates of hydroxyethyl starch) weighed 8.1 g.

Sulphur content: 17.2%,

$$[\eta]_{H_2O}^{30}=0.034.$$

Example 7

1 g of sodium salts of sulphates of hydroxyethyl starch (sulphur content=13.17%) were dissolved in 10 ml of water, and then 3.5 ml of methanol were added.

0.79 gram of $AlCl_3.6H_2O$ was dissolved in 4 ml of water and to the solution 1.6 g of 7% NH_3 aqueous solution were slowly added. Basic aluminium chloride aqueous solution was produced. This solution was added to the above-mentioned solution of esterified sodium salt of the sulphate of hydroxyethyl starch with stirring; the stirring was continued at 25°C for 3.5 hours. The resulting precipitate was separated and dispersed in 10 ml of water to which 5 ml of methanol were added. The precipitate was separated, washed with alcohol and dried at under 80°C. The aluminium salts of sulphates of hydroxyethyl starch thus obtained weighed 0.83 g.

Example 8

1 g of sodium salts of sulphates of hydroxyethyl starch (sulphur content=13.17%) was dissolved in 50 ml of 50% aqueous glycerine solution.

2.5 g of $Bi(NO_3)_3.5H_2O$ were dissolved in 25 ml of water. This solution was added to the above-mentioned solution of esterified sodium salts of sulphates of hydroxyethyl starch. The pH of the resulting solution was adjusted to 4.5, and the precipitate which formed was separated by centrifuging and dispersed in 10 ml of water. The precipitate was separated, washed with alcohol and dried. The bismuth complex of the sulphates of hydroxyethyl starch which was obtained weighed 0.98 g.

Example 9

1 g of sodium salts of sulphates of hydroxyethyl starch (sulphur content=13.17%) was dissolved in 20 ml of water and to the solution a cation exchange resin was added. The mixture was stirred at room temperature for 5 hours to convert the salt form to the free acid form of sulphate of hydroxyethyl starch, and to the mixture 400 mg of ferrous sulphate were added with stirring. The solution thus obtained was passed through an anion exchange resin column. The effluent was freeze-dried. 0.78 g of the ferrous salt of the sulphate of hydroxyethyl starch was obtained.

Example 10

The initial part of the procedure described in Example 1 was repeated, and the 850 ml solution obtained by combining the filtrate and the washing solution was mixed with 300 ml of ethanol. The mixture was allowed to stand overnight. After filtration of the mixture, the filtrate was concentrated using a "Diafilter" as described above. The filtrate was concentrated to 100 ml. After freeze-drying, 12.81 g of calcium salts of sulphates of hydroxyethyl starch were obtained.

Example 11

8 ml of chlorosulphonic acid were added dropwise to 420 ml of pyridine at below 10°C. The precipitated pyridinium salt of chlorosulphonic acid was melted by heating. To the melted salt, were added in small portions 50 g of hydroxyethyl starch (substitution degree of hydroxyethyl group 0.920). The mixture was stirred at 70°C for 45 minutes, and then 500 ml of water were added to stop the reaction. Acetone was added to the mixture in a ratio of 2 litres of acetone per litre of the aqueous solution.

A large amount of an oily precipitate was produced, and the supernatant liquid was removed. Water was added to the oily precipitate in a ratio of 400 ml of water per 100 ml of precipitate. The pH of the solution was 5.58. The mixture was made alkaline by the addition of 48 ml of a saturated aqueous solution of Na_2CO_3, which brought the pH to 7.72. Ethanol was added to the solution in the ratio of 1.2 litres of ethanol per 600 ml of solution to form a precipitate, and the mixture was left standing overnight at room temperature.

The supernatant was separated from the mixture, and ethanol was added to harden the precipitate. The precipitate was powdered in a Waring blender. The mixture was filtered and the precipitate washed successively with ethanol and ether. 38.2 grams of sodium salts of sulphates of hydroxyethyl starch were obtained.

35 grams of the thus obtained sodium salts of sulphates of hydroxyethyl starch were dissolved in 250 ml of water, and the resulting solution was placed in a "Diafilter" acting as a sieve allowing the passage therethrough of compounds having a molecular weight of up to 30,000, and washed with

water, de-salted and concentrated, as in previous Examples. 250 ml of the solution within the "Diafilter" were freeze-dried, to yield 25.3 g of sodium salts of sulphates of hydroxyethyl starch.

Sulphur content: 4.25%

$$[\eta]_{H_2O}^{30}=0.42.$$

Example 12

150 ml of conc. sulphuric acid were cooled to form a paste. To the sulphuric acid were added in small portions over a period of 15 minutes at −25°C, 50 g of hydroxyethyl starch (substitution degree of hydroxyethyl group=0.92), and the mixture was stirred for an additional 15 minutes as it warmed to 0°C. Thereafter the mixture was stirred for an additional 4 hours. During this period the mixture became brown.

The mixture and 180 ml of cooled diethyl ether were mixed at −30°C. A brown precipitate was separated by filtration through a glass filter, and washed with cooled diethyl ether. The precipitate was dissolved in 200 ml of water, and the ethereal phase removed. The pH of a brown solution was 0.21. The pH of the solution was adjusted with 46 ml of 20% NaOH aqueous solution to 7.06. To 350 ml of the neutralized solutions were added 1.2 litres of acetone. The initial oily precipitate became flaky on standing. The supernatant was removed, and ethanol was added to the precipitate. The powdered precipitate was separated by filtration, washed successively with ethanol and ether, and dried. The resulting white powder of sodium salts of sulphates of hydroxyethyl starch weighed 42.4 g.

Sulphur content: 13.46%,

$$[\eta]_{H_2O}^{30}=0.038.$$

Example 13

Using 150 ml of conc. sulphuric acid and 50 g of hydroxyethyl starch (substitution degree of hydroxyethyl group=0.92), a reaction was carried out and the reaction mixture was treated in the same manner as that described in Example 12. 67.8 grams of brown sodium salts of sulphates of hydroxyethyl starch were obtained.

Sulphur content: 15.48%,

$$[\eta]_{H_2O}^{30}=0.032.$$

Example 14

Using 75 ml of conc. sulphuric acid and 50 g of hydroxyethyl starch (substitution degree of hydroxyethyl group=0.92), a reaction was carried out and the reaction mixture was treated in the same manner as that described in Example 12. 35.2 grams of brown sodium salts of sulphates of hydroxyethyl starch were obtained.

Sulphur content: 11.84%,

$$[\eta]_{H_2O}^{30}=0.040.$$

Example 15

100 grams of sodium salts of sulphates of hydroxyethyl starch obtained as in Example 12 were added to 1000 ml of distilled water. A clear solution was obtained.

In a similar manner, solutions of various concentrations of sodium salts of sulphates of hydroxyethyl starch, were prepared for oral or parenteral administration.

Example 16

10 Kg of sodium salts of sulphates of hydroxyethyl starch obtained by the procedure of Example 12, 7.5 kg of corn starch, 4 kg of talc and 0.13 kg of magnesium stearate were thoroughly mixed, and 50,000 capsules were charged with equal amounts of the resulting mixture in the usual manner. The capsules were employed as anti-inflammatory agents, by the oral administration of 1 or 2 capsules every 6 to 12 hours.

Example 17

10 Kg of sodium salts of sulphates of hydroxyethyl starch obtained by the procedure of Example 12, 2.5 kg of lactose, 1.5 kg of corn starch, 0.15 kg of magnesium stearate and 0.06 kg of light liquid paraffin were thoroughly mixed and slugged. The slugs were forced through a screen, and the resulting granules were then compressed into 100,000 tablets, each tablet containing 100 mg of the active ingredient.

Example 18

A sterile aqueous solution for intramuscular injection was prepared from 100 g of sodium salts of sulphates of hydroxyethyl starch obtained by the procedure of Example 13 and 1000 ml of water. The active compound was dissolved in the water, and sufficient sodium hydroxide was added to form a solution with a pH of 7.2. The solution was sterilized by filtration. 1 Ml batches of the solution were introduced into sterile vials and lyophilized, whereupon the vials were sealed. Immediately prior to use, sufficient sterile water for injection to make 1 ml of solution was added to each vial.

Example 19

Preparation of tablets

Prescription:

Sodium sulphate of hydroxyethyl starch obtained as in Example 12	2 g

Example 19 (cont.)

Lactose	0.4 g
Starch	0.48 g
Talc	0.1 g
Magnesium stearate	0.02 g

The whole was made into 20 tablets by means of a 10 mm-deep cup punch.

The tablets were coated in a conventional manner.

Example 20

Sodium salts of sulphates of hydroxyethyl starch obtained by the procedure of Example 13, were dissolved in isotonic sodium chloride solution. The resulting solution was sterilized by passage through a microporous filter, and single doses of varying strength were injected intraperitoneally (0.5 ml solution per 100 g of body weight) in a standard toxicity test into male mice weighing 16.0—20.0 g each. The mean lethal dosage (LD_{50}) was determined two weeks after the injection according to Litchfield-Wilcoxon method. The result obtained was $LD_{50}=4.61$ g/kg.

Example 21

Sodium salts of sulphates of hydroxyethyl starch obtained as described in Examples 1 to 14 were dissolved in distilled water. The resulting solution was sterilized by passage through a microporous filter, and single doses of varying strength were administered orally (0.5 ml solution per 10 g of body weight) in a standard toxicity test to male mice weighing 17.0—20.0 g each. The mean lethal dosage (LD_{50}) was determined two weeks after the injection, according to the Litchfield-Wilcoxon method. The result obtained was $LD_{50}>10.0$ g/kg.

WHAT WE CLAIM IS:—

1. A sulphate of hydroxyethyl starch, in which the sulphate is in free acid form.

2. A sulphate of hydroxyethyl starch, in which the sulphate is in the form of a metal salt or a metal complex.

3. A sulphate of hydroxyethyl starch according to Claim 2, wherein the metal is selected from sodium, potassium, magnesium, calcium, aluminium, bismuth and iron.

4. A sulphate of hydroxyethyl starch according to Claims 1, 2 or 3, wherein the molecular weight of the hydroxyethyl starch moiety is in the range from 1000 to 200,000, the degree of substitution of hydroxyethyl groups per anhydroglucose unit in the hydroxyethyl starch is in the range from 0.2 to 1.2, the sulphur content is in the range from 2 to 18% by weight of the sulphate of hydroxyethyl starch, and the intrinsic viscosity of the sulphate is in the range from 0.02 to 0.5 at 30°C in water.

5. A process for producing a sulphate of hydroxyethyl starch as claimed in Claims 1, 2 or 3, which comprises reacting hydroxyethyl starch either with chlorosulphonic acid in pyridine, or with sulphuric acid; neutralizing the reaction solution; filtering the solution; mixing the resulting filtrate with a water-miscible organic solvent; separating the resulting precipitate; and, if necessary, converting the sulphate from free acid form to metal salt or metal complex form.

6. A process according to Claim 5, wherein the hydroxyethyl starch is one prepared by contacting waxy corn starch having an amylopectin content of over 98% by weight, with ethylene oxide or ethylene chlorohydrin.

7. A process according to Claim 5 or 6, wherein the molecular weight of the hydroxyethyl starch is in the range from 1000 to 200,000, and the degree of substitution of hydroxyethyl groups per anyhydroglucose unit in the hydroxyethyl starch is in the range from 0.2 to 1.2.

8. A process according to Claims 5, 6 or 7, wherein the filtrate is concentrated prior to being mixed with the water-miscible organic solvent.

9. A process according to any one of Claims 5 to 8, wherein the water-miscible organic solvent is methanol, ethanol or acetone.

10. A process according to any one of Claims 5 to 9, wherein the neutralization is effected with sodium carbonate.

11. A process according to any one of Claims 5 to 10, wherein, when the hydroxyethyl starch is reacted with sulphuric acid, from 20 to 50 grams of hydroxyethyl starch are employed per 100 ml of combined hydroxyethyl starch and sulphuric acid.

12. A process according to any one of Claims 5 to 10, wherein, when the hydroxyethyl starch is reacted with chlorosulphonic acid in pyridine, there are employed from 0.5 to 3 moles of chlorosulphonic acid per mole of anhydroglucose units of hydroxyethyl starch.

13. A process according to any one of Claims 5 to 10 and 12, wherein, when the hydroxyethyl starch is reacted with chlorosulphonic acid in pyridine, there are employed 3 to 10 ml of pyridine per gram of hydroxyethyl starch.

14. A process according to Claim 5, substantially as described in any of the foregoing Examples 1 to 14.

15. A sulphate of hydroxyethyl starch,

whenever produced by a process according to any one of Claims 5 to 14.

16. A sulphate of hydroxyethyl starch, substantially as described in any one of the foregoing Examples 1 to 14.

17. A pharmaceutical composition comprising at least one sulphate of hydroxyethyl starch as claimed in any one of Claims 1 to 4, 15 and 16; and a pharmacutically-acceptable diluent or carrier therefor.

18. A pharmaceutical composition substantially as described in any one of the foregoing Examples 15 to 21.

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